MASSACHUSETTS STATE POLICE FORENSIC SERVICES GROUP

TOXICOLOGY UNIT

IDENTIFICATION, QUANTIFICATION, AND CONFIRMATION OF GHB BY GC-MS

Version 4.0

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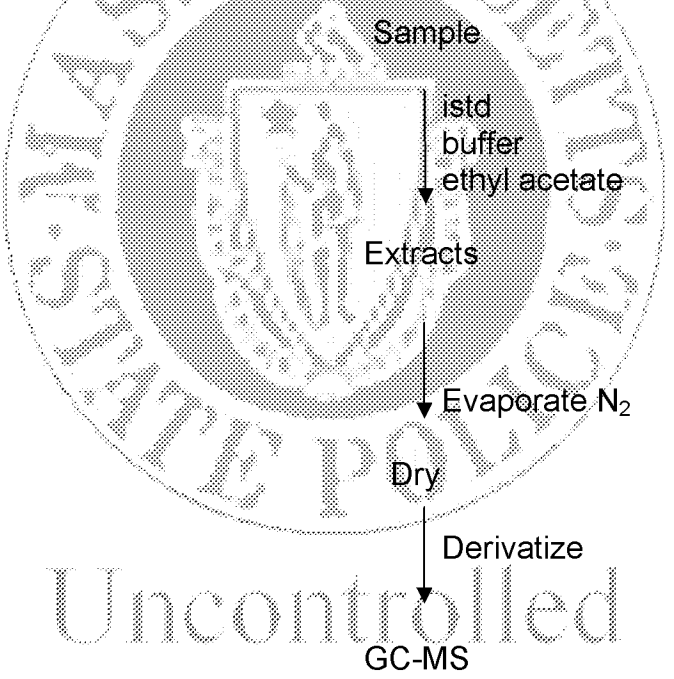
1 INTRODUCTION

A confirmation procedure for the identification, and quantification of Gamma-hydroxybutyric acid (GHB) in samples (urine, blood, and liquids). This method is unique in that it does not involve the conversion of GHB to the gamma-butyrolactone (GBL). In summary, to all samples the internal standard GHB-d_6 is added, and then buffered and extracted using ethyl acetate, evaporated and derivatized with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS), and analyzed by GC-MS. Quantification was performed using selective ion monitoring (SIM), using GHB-d_6 as the internal standard.

1.1 Flow Diagram



1.2 Reagents and Equipments

1.2.1 All reagents must be ACS grade or better.

1.2.1.1	Ammonium chloride	Fisher A661-500, or equivalent
1.2.1.2	Ethyl Acetate	Fisher E-189, or equivalent
1.2.1.3	Acetonitrile	Fisher A-998, or equivalent

1.2.1.4	BSTFA+TMCS, 99:1	Supelco 3-3148, or equivalent
1.2.1.5	GHB-d_6	Cerilliant G-003, or equivalent
1.2.1.6	GHB	Sigma H-3635, or equivalent
1.2.1.7	Deionized water	
1.2.1.8	Sulfuric Acid	J.T. Baker, or equivalent

1.2.2 Reagents preparation

1.2.2.1 Saturated ammonium chloride, Add ammonium chloride to 10 ml to deionized water to a point of saturation.

1.2.3 Equipment

1.2.3.1	GC-MS	HP GC 6890 and MSD 5975, equipped with 7683 HP automatic injector or equivalent
1.2.3.2	Capillary column	15 m length, 0.25 mm ID, 0.25 μm thickness, HP-5 or equivalent
1.2.3.3	Test tubes Scientific	13-mm borosilicate glass, Fisher or equivalent
1.2.3.4	Test tubes Scientific	12-mm borosilicate glass, Fisher or equivalent
1.2.3.5	Pipettor	Eppendorf variable (100-1000), (10-100), (2-20), and (0.1-2.5), or equivalent.
1.2.3.6	Centrifuge	IEC HN-SII or equivalent
1.2.3.7	Heating module	Pierce Reacti-Therm 18800 or equivalent
1.2.3.8	Orbitron	Boekel or equivalent
1.2.3.9	Glass vials	National Scientific Co. C4000-1W or equivalent

1.2.3.10 Glass insert — National Scientific Co. C4010-630 or equivalent

1.2.3.11 Cap and septa — National Scientific Co. C4000-5313 or equivalent

1.3 Conditions

1.3.1 Injector temperature — 280°C

1.3.2 Detector temperature — 300°C

1.3.3 Programmed temperature

1.3.3.1 Initial — 80°C hold for 2.0 minute

1.3.3.2 Rate — 30°C/Minutes

1.3.3.3 Final — 300°C hold for 1.0 minute

1.3.4 Ions monitored during the run

Compounds	Quantification ion (m/z)	Qualification ions (m/z)
GHB-d_6	239	240,241
GHB	233	234,235

1.3.5 When other ions are used, it should be noted in the case file.

2 STANDARDS AND INTERNAL STANDARD

2.1 Standards

2.1.1 Stock Standards

2.1.1.1 GHB — 10 mg/dl in methanol or equivalent

2.1.2 Working standards

2.1.2.1 GHB 1.0 mg/dl; using a 10ml volumetric flask, add 1.0ml of GHB (10mg/dl) stock solution (2.1.1.1), and dilute to mark with acetonitrile

2.2 Internal Standards

2.2.1 Stock Internal Standard

2.2.1.1 GHB-d_6 10 mg/dl as sodium salt in methanol or equivalent

2.2.2 Working Internal Standard

2.2.2.1 GHB- d_6 1.0mg/dl as sodium salt; using a 10ml volumetric flask, add 1.0 ml of GHB-d_6 (10 mg/dl) stock solution (2.2.1.1) and dilute to mark with acetonitrile

3 QUALITY CONTROL

3.1 Controls

3.1.1 Controls are either prepared from the reference material, separately from stock standards (2.1.1), weighed or measured separately, purchased, or obtained from previously analyzed samples.

3.2 Stock controls

3.2.1 GHB 10 mg/dl in methanol or equivalent

3.3 Working controls

3.3.1 GHB 1.0 mg/dl; using a 10ml volumetric flask, add 1.0ml of GHB (10mg/dl) stock solution (3.1.1), and dilute to mark with acetonitrile

3.3.2 With each batch of specimens, controls would be carried through the procedure in parallel with the unknown.

3.3.3 It is suggested that each batch of specimens include at least 10% controls with the minimum of one positive and one negative control.

4 PROCEDURE

4.1 Calibrators preparation

4.1.1 To the appropriate 13-mm test tube, add the following (2.1.2) working standard.

Final calibrator concentration	Working standard	Deionized water
500μg/dl	25 μl	25 μl
1000μg/dl	50 μl	0 μl
2000μg/dl	100 μl	0 μl

4.2 Controls preparation

4.2.1 To the appropriate 13-mm test tube, add the following (2.2.2) working standard.

4.2.2 Each batch of specimens includes 10% controls with the minimum of one positive and one negative control.

Final control concentration	Working control	Deionized water/matrix-match
Blank	0 μl	50 μl
1000μg/dl	50 μl	0 μl

4.2.3 Add 50μl of the unknown specimen, in 13-mm test tube.

4.2.4 Add 0.5 ml saturated ammonium chloride (1.2.2.1) to all tubes.

4.2.5 Add 30μl (2.2.2) working internal standard to all tubes.

4.2.6 Add 3ml ethyl acetate to all tubes.

4.2.7 Mix all tubes on orbitron for approximately 5 minutes.

4.2.8 Centrifuge all tubes at 3000 rpm for approximately 5 minutes.

4.2.9 Transfer the organic phase to 12-mm test tubes.

4.2.10 Evaporate to dryness at room temperature under a gentle stream of Nitrogen.

4.3 Derivatization

4.3.1 Add 20μl of BSTFA+TMCS 99:1(1.2.1.4).

4.3.2 Add 60µl of ethyl acetate.

4.3.3 Cap and mix.

4.3.4 Derivatize at approximately 70°C for 20 minutes using (1.2.3.7) Heating module, Pierce Reacti-Therm 18800 or equivalent.

4.3.5 Remove from heating module and allow to cool to room temperature.

4.4 Preparation for GC-MS

4.4.1 Transfer to auto-sample vial and seal.

4.4.2 Inject 2 µl into the GC-MS.

4.5 Calculation of the calibrators curve

4.5.1 The calibrators curve is generated by the software, using all 3 calibrators.

4.5.2 Response of peak-area ratios of GHB to the GHB-d_6 (internal standard) are set up for each concentration.

4.5.3 The calibrator curve is then generated for GHB, and all results are calculated using this new curve.

4.5.4 The calculations use a linear fit.

4.5.5 Ion 233 m/z is used for the calculations of GHB, and ion 239 m/z for GHB-d_6.

4.5.6 All other ions are used as qualifiers.

4.5.7 The correlation coefficient of the calibrators' curves must be ≥0.98 for acceptance.

4.5.8 The qualifier ion-ratio must be within ±20% of the 1000µg/dl calibrator that the software uses to calculate the qualifiers.

4.5.9 Make all the necessary adjustments to the calculations (e.g. multiply using the appropriate dilution factor) if less than 50µl of sample is tested.

5 RESULTS

5.1 Results

5.1.1 The retention time must be within ±2% or 0.2 minutes of the 1000µg/dl calibrator.

5.1.2 If the concentration of the specimen exceeds the concentration of the highest calibrator, the specimen should be diluted and re-extracted.

5.1.3 The results must have the quantitative and the qualitative ion-ratios within ±20% of the 1000µg/dl calibrator.

5.1.4 The cut-off for blood is 500µg/dl, and for urine is 1000µg/dl.

5.1.5 Screening for GHB can be based on running a positive control and a negative control only. The positive control is equivalent to the low calibrator.

5.2 Interpretation of Results

5.2.1 Results will only be reported only when the following parameters are met.

5.2.1.1 Quantitative and qualitative ion-ratios must be within the acceptable range (4.6.9).

5.2.1.2 Retention times must be within the acceptable range (5.1.1).

5.2.1.3 Quality control specimen results are within ±20% of the expected quantitative value.

5.2.2 Any deviation from this method must be noted in the case file and should be approved by the Lead Supervisor or section designee.

6 QUALITY CONTROL CRITERIA

6.1 Criteria

6.1.1 Results must fall within ±20% of the targeted value.

6.1.2 All results are recorded.

7 LIMITATIONS

7.1 Defines

7.1.1	Limit of quantification	500μg/dl or the lowest calibrator
7.1.2	Limit of detection	200μg/dl
7.1.3	Linearity	200-10000 μg/dl

7.1.4 Since there is no screening method for GHB, all positive results must be re-run with a second aliquot of the same specimen.

8 SAFETY PRECAUTION

8.1 Safety

8.1.1 All biological specimens must be considered pathogenic.

8.1.2 All biological specimens must be handled wearing gloves.

8.1.3 Derivatization step (4.4) must be carried out in the hood.

8.1.4 Refer to the lab safety manual for additional precautions.

9 REFERENCE

Elian, A.A., "A novel method for GHB detection in urine and its application in drug-facilitated sexual assaults" Forensic Science International, 109, April 2000, pp.183-187

10 REVISION HISTORY

REVISION DATE	VERSION	Approval: Forensic Services Group Director	TOTAL PAGES	REVISION
9/00/2000	1.0	Al Elian, Ken Gagnon, Carl Selavka	6	Original
9/00/2001	2.0	Al Elian, Ken Gagnon, Carl Selavka	6	.
2/28/2007	3.0	Ken Gagnon, Carl Selavka	6	Update GC-MS method.
8/27/2009	4.0	Al Elian, Ken Gagnon, Major James Connolly	12	Format revision.

11 MANUAL AUTHORIZATION

APPROVED BY: ON FILE IN THE QUALITY ASSURANCE OFFICE

FORENSIC SERVICES GROUP DIRECTOR	**DATE**
LEAD SUPERVISOR FORENSIC CHEMISTRY	**DATE**
TECHNICAL LEADER FORENSIC CHEMISTRY	**DATE**

REVIEWED BY:

QUALITY ASSURANCE LEADER CRIME LABORATORY SYSTEMS	**DATE**